



## Exploratory study of the effect of one week of orally administered CNSA-001 (sepiapterin) on CNS levels of tetrahydrobiopterin, dihydrobiopterin and monoamine neurotransmitter metabolites in healthy volunteers

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**Abstract:** Tetrahydrobiopterin (BH4) is a cofactor for the enzymes tyrosine hydroxylase and tryptophan hydroxylase, the rate-limiting enzymes in the production of the neurotransmitters, dopamine and serotonin, respectively, in the central nervous system (CNS). Administration of BH4 is used clinically within the management of persons with genetic BH4 deficiencies, but the BH4 molecule does not cross the blood-brain barrier sufficiently. CNSA-001 is a pharmaceutical preparation of sepiapterin, a natural precursor of BH4 that induced larger increases in plasma BH4 compared with administration of the same doses of BH4 itself in healthy volunteers in a randomized trial. Here, we report the effects of 7 days of once-daily treatment with CNSA-001 60mg/kg (n=6) or placebo (n=2) on metabolites of the BH4 synthetic pathway and on biomarkers of the serotonin (5-hydroxyindoleacetic acid [5-HIAA]) and dopamine (homovanillic acid [HVA]) pathways in cerebrospinal fluid (CSF) in subjects from this trial. There were no notable changes in any metabolite in placebo-treated subjects. Administration of CNSA-001 increased mean BH4 from 18.1 (SD 3.0) to 35.1 (10.0) nmol/L, and of dihydrobiopterin (BH2) from 2.1 (0.3) to 7.9 (1.5) nmol/L. Overall, administration of CNSA-001 had little effect on mean levels (pre- vs. post-treatment) of 5-HIAA (76.1 [SD 29.8] vs. 70.1 [23.1] nmol/L) or HVA (177.2 [66.5] vs. 184.8 [35.3]) nmol/L. One subject with low 5-HIAA and HVA at baseline responded with approximately three-fold increases in CNS levels of these metabolites after CNSA-001 treatment, with post-treatment levels within the range of those seen in other subjects. Administration of CNSA-001 60mg/kg markedly increased levels of BH4 in the CNS of healthy volunteers, with apparently little overall effect in CNS levels of already normal key neurotransmitter metabolites.

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# Exploratory study of the effect of one week of orally administered CNSA-001 (sepiapterin) on CNS levels of tetrahydrobiopterin, dihydrobiopterin and monoamine neurotransmitter metabolites in healthy volunteers



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## ABSTRACT

Tetrahydrobiopterin (BH<sub>4</sub>) is a cofactor for the enzymes tyrosine hydroxylase and tryptophan hydroxylase, the rate-limiting enzymes in the production of the neurotransmitters, dopamine and serotonin, respectively, in the central nervous system (CNS). Administration of BH<sub>4</sub> is used clinically within the management of persons with genetic BH<sub>4</sub> deficiencies, but the BH<sub>4</sub> molecule does not cross the blood-brain barrier sufficiently. CNSA-001 is a pharmaceutical preparation of sepiapterin, a natural precursor of BH<sub>4</sub> that induced larger increases in plasma BH<sub>4</sub> compared with administration of the same doses of BH<sub>4</sub> itself in healthy volunteers in a randomized trial. Here, we report the effects of 7 days of once-daily treatment with CNSA-001 60 mg/kg (*n* = 6) or placebo (*n* = 2) on metabolites of the BH<sub>4</sub> synthetic pathway and on biomarkers of the serotonin (5-hydroxyindoleacetic acid [5-HIAA]) and dopamine (homovanillic acid [HVA]) pathways in cerebrospinal fluid (CSF) in subjects from this trial. There were no notable changes in any metabolite in placebo-treated subjects. Administration of CNSA-001 increased mean BH<sub>4</sub> from 18.1 (SD 3.0) to 35.1 (10.0) nmol/L, and of dihydrobiopterin (BH<sub>2</sub>) from 2.1 (0.3) to 7.9 (1.5) nmol/L. Overall, administration of CNSA-001 had little effect on mean levels (pre- vs. post-treatment) of 5-HIAA (76.1 [SD 29.8] vs. 70.1 [23.1] nmol/L) or HVA (177.2 [66.5] vs. 184.8 [35.3] nmol/L). One subject with low 5-HIAA and HVA at baseline responded with approximately three-fold increases in CNS levels of these metabolites after CNSA-001 treatment, with post-treatment levels within the range of those seen in other subjects. Administration of CNSA-001 60 mg/kg markedly increased levels of BH<sub>4</sub> in the CNS of healthy volunteers, with apparently little overall effect in CNS levels of already normal key neurotransmitter metabolites.

## 1. Introduction

Tetrahydrobiopterin (BH<sub>4</sub>) is an essential cofactor for the enzyme, phenylalanine hydroxylase, as well as hydroxylases that perform the rate limiting step in the synthesis of monoamine neurotransmitters in the central and peripheral nervous systems (tyrosine hydroxylase and tryptophan hydroxylase) [1–3]. Subjects with defects in BH<sub>4</sub> synthesis often present with symptoms consistent with reduced function of these neurotransmitter systems and may benefit from treatment with the products of these hydroxylases (levodopa and 5-hydroxytryptophan) [4–6].

Increasing levels of BH<sub>4</sub> within the brain would represent an alternative approach to the management of CNS symptoms in patients with

BH<sub>4</sub> deficiencies. However, BH<sub>4</sub> itself does not cross the blood brain barrier sufficiently, which limits the potential efficacy of this approach [7,8]. Administration of other components of the BH<sub>4</sub> synthetic pathway may provide an alternative approach. CNSA-001 is a novel formulation of sepiapterin, a natural precursor of BH<sub>4</sub>. Oral administration of CNSA-001 to healthy volunteers has been shown to induce larger increases in plasma levels of BH<sub>4</sub>, compared with administration of equivalent doses of BH<sub>4</sub> itself, in healthy subjects in a randomized clinical study [9]. We describe an exploratory study that evaluated the effects of oral CNSA-001, a pharmaceutical formulation of sepiapterin, on levels of BH<sub>4</sub>, dihydrobiopterin (BH<sub>2</sub>, an intermediate in BH<sub>4</sub> metabolism) and metabolites of serotonin (5-hydroxyindoleacetic acid [5-HIAA]) and dopamine (homovanillic acid [HVA]) in cerebrospinal fluid

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(CSF) in healthy volunteers.

## 2. Methods

The data presented here are the results of an exploratory analysis in one study cohort from a randomized, double-blind Phase I evaluation of the pharmacokinetics of CNSA-001 in healthy volunteers. The study design and main results of this study are described in detail elsewhere [9]. Briefly, subjects eligible for inclusion were adult ( $\geq 18$  y) men or non-pregnant women (55–100 kg), who were not taking antifolate medication and who were free of significant chronic illness or gastrointestinal disorders, and had not smoked tobacco for the previous two weeks. Women were required to maintain adequate contraception. Informed consent was obtained from participants and ethical requirements for the conduct of clinical research in humans were met in full.

Measurements in CSF were made from subjects who received oral treatment with CNSA-001 60 mg/kg/day ( $n = 6$ ) or placebo ( $n = 2$ ) once daily for seven days. The placebo used for this study was a formulated suspension containing identical excipients as found in the drug product formulation of CNSA-001 with the exception of substitution of microcrystalline cellulose for active ingredient and the addition of yellow dye #6 to match color. Cerebrospinal fluid (CSF) was obtained by lumbar puncture predose on day 1 and again following treatment on Day 7 at the projected plasma  $T_{max}$  for BH<sub>4</sub>. All lumbar punctures were performed at the same time of day. All measurements of metabolites of interest were conducted at MNG Laboratories, Atlanta, GA, USA. Measurements of BH<sub>4</sub> and BH<sub>2</sub>, were made using high performance liquid chromatography/mass spectrometry [9]. The concentrations of neopterin in CSF were determined using high performance liquid chromatography followed by sequential electrochemical and fluorescence detection [10]. Concentrations in CSF of 5-HIAA and HVA were measured following HPLC separation by electrochemical detection [11]. Normal reference ranges used by this laboratory for metabolites of interest in adults are: 5-HIAA 67–140 nmol/L; HVA 145–324 nmol/L; BH<sub>4</sub> 10–30 nmol/L; neopterin 8–28 nmol/L. Methodologies for measuring pterins are still evolving, and reference ranges for BH<sub>2</sub> are not available currently. Nevertheless, data on BH<sub>2</sub> are included for completeness.

## 3. Results

Administration of CNSA-001 60 mg/kg/day for 7 days led to an approximate doubling of levels of BH<sub>4</sub>, an approximate tripling of levels of BH<sub>2</sub>, and no change in neopterin concentration in CSF, with little or no change caused by placebo (Table 1, Fig. 1). The BH<sub>4</sub>:BH<sub>2</sub> ratio decreased, but there were only minor or no changes in the mean levels of 5-HIAA and HVA following treatment with CNSA-001 or placebo (Table 1).

Fig. 1 shows medians, interquartile ranges and maximum and minimum values of BH<sub>4</sub>, BH<sub>2</sub>, HVA and 5-HIAA before and after administration of CNSA-001 for one week. Interestingly, pre-dose HVA and 5-HIAA concentrations in one subject were about one-third of that observed in the other subjects (55 nmol/L and 19 nmol/L, respectively), but increased to 164 nmol/L and 50 nmol/L, respectively, which was within the range of values in the other subjects.

## 4. Discussion

The main finding of this exploratory study was that oral administration of CNSA-001 for one week induced marked increases in the levels of BH<sub>4</sub>, along with its precursor, BH<sub>2</sub>, in the CSF of healthy volunteers. In effect, CNSA-001 appeared to act as a prodrug for BH<sub>4</sub> in this setting.

Our study population was composed of healthy subjects with no signs or symptoms of neurotransmitter deficiency. There was little effect of CNSA-001 on mean values of HVA or 5-HIAA. This implied a lack

**Table 1**

Exploratory data from measurements in cerebrospinal fluid predose and following seven days of administration of CNSA-001 or placebo.

	CNSA-001 60 mg/kg/day ( $n = 6$ )	Placebo <sup>a</sup> ( $n = 2$ )
Mean BH <sub>4</sub> nmol/L (SD)		
Baseline	18.1 (3.0)	12.8; 13.5
Day 7	35.1 (10.0)	13.5; 13.1
Mean change from baseline (SD)	17.0 (12.2)	
Mean BH <sub>2</sub> nmol/L (SD)		
Baseline	2.1 (0.3)	2.5; 1.5
Day 7	7.9 (1.5)	2.5; 1.5
Mean change from baseline (SD)	5.7 (1.5)	
Mean 5-HIAA nmol/L (SD)		
Baseline	76.1 (29.8)	49.6; 75.0
Day 7	70.1 (23.1)	43.7; 55.5
Mean change from baseline (SD)	−6.0 (24.0)	
Mean HVA nmol/L (SD)		
Baseline	177.2 (66.5)	123.2; 152.5
Day 7	184.8 (35.3)	128.7; 140.2
Mean change from baseline (SD)	7.6 (65.8)	
Mean neopterin nmol/L (SD)		
Baseline	15.0 (4.1)	25.0; 12.0
Day 7	14.9 (3.7)	24.6; 10.2
Mean change from baseline (SD)	−0.04 (1.6)	

Treatments were given for 7 days. Measurements on day 7 were made  $\pm$  30 min from the time of BH<sub>4</sub>  $T_{max}$ . Measurements were made in 6 and 2 subjects randomly assigned to CNSA-001 and placebo, respectively. 5-HIAA: 5-hydroxyindoleacetic acid; HVA: homovanillic acid.

<sup>a</sup> Single values.

of overall effect of CNSA-001 on the activity of CNS pathways mediated by serotonin or dopamine, despite the approximate doubling in BH<sub>4</sub> concentration in the CSF. Alternatively, this provides evidence that prolonged exposure to increased concentrations of BH<sub>4</sub> and BH<sub>2</sub> in the CSF does not enhance the metabolism of monoamines in healthy persons with normal concentrations of neurotransmitters and normal functioning dopamine and serotonin regulation. These findings were consistent with those of the overall study, in which no adverse events suggestive of neurotransmitter disturbances were observed [9].

The marked increase in 5-HIAA and HVA in one subject with abnormally low levels of these metabolites at baseline was interesting, as treatment with CNSA-001 appeared to normalize the concentrations of these metabolites in this patient's CSF. BH<sub>4</sub> levels in CSF in this subject also responded markedly to treatment with CNSA-001. Indeed, the BH<sub>4</sub> level in the CSF of this subject was lower than in any other subject at baseline, and higher than any other subject after treatment.

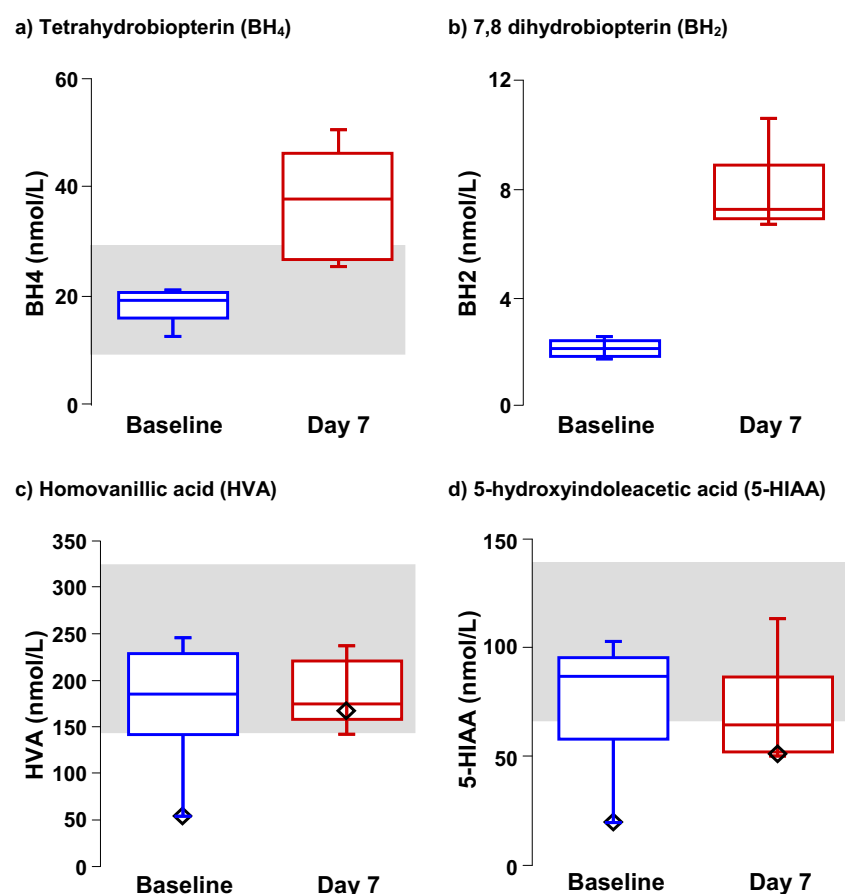
The small size of this exploratory study was its most obvious limitation, particularly as only two subjects received placebo. Our results must be interpreted with caution, although sepiapterin and placebo were administered in a prospective, double-blind, randomized manner.

## 5. Conclusions

Oral administration of CNSA-001, a pharmaceutical formulation of sepiapterin, induced marked increases in the level of BH<sub>4</sub> in CSF, with no overall effect on the levels of metabolites of serotonin and dopamine when given to healthy volunteers. Further study will be required to evaluate the effects of CNSA-001 on CNS neurotransmitter function in patients with BH<sub>4</sub> deficiencies.

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**Fig. 1.** Exploratory data from measurements of pterins and biogenic amines, before and after 7 days of CNSA-001 (sepiapterin) administration, in cerebrospinal fluid (CSF) from healthy volunteers. Bars show maximum and minimum values; boxes show median and interquartile ranges. Diamonds show values of HVA and 5-HIAA from one subject who had unusually low levels of these metabolites in CSF at baseline but responded with substantial increases in these metabolites after treatment with CNSA-001. Shaded areas show laboratory reference ranges for each metabolite (not available for BH<sub>2</sub>, see text).

this article.

#### Declaration of Competing Interest

- NS is an employee and stockholder of Censa Pharmaceuticals Inc., the pharmaceutical sponsor of the investigational treatment evaluated in this study.
- NB reports consultancy for and stock options in Censa Pharmaceuticals.
- NL performs clinical trials and is a consultant for Censa.
- KH and KL are employees of MNG Laboratories, LLC.
- KH reports consultancy for and stock options in Censa Pharmaceuticals.

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